

WO 2004/008138

PCT/DK2003/000483

43

CLAIMS

1. An extracorporeal adsorption method for removing harmful substances responsible of inducing sepsis caused by Gram-negative in a mammal, said extracorporeal adsorption method being effected by an adsorption column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for the LPS portion of said Gram-negative bacteria, said method comprising treating blood obtained from said mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed.
2. An extracorporeal adsorption method for removing harmful substances responsible of inducing sepsis caused by Gram-negative or Gram-positive bacteria in a mammal, said extracorporeal adsorption method being effected by an adsorption column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for:
 - i) the LPS portion of said Gram-negative bacteria, and/or
 - ii) Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria.
3. A method according to claim 1 or 2 wherein the treated blood is capable of being reinfused into the same mammal.
4. A method according to any of claims 1-3, wherein the adsorption column assembly is adapted for fluidised bed adsorption, in particular stabilised fluidised bed adsorption.
5. A method according to any of the preceding claims, wherein the particles have a density of at least 1.3 g/ml and a mean diameter in the range of 5-1000 µm, such as a density of at least 1.5 g/ml and a mean diameter in the range of 5-300 µm, preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 µm, and most preferred a density of more than 2.5 g/ml and a mean diameter in the range of 5-75 µm.

35

6. A method according to any of the preceding claims, wherein the mammal is a human being.

WO 2004/008138

PCT/DK2003/000483

44

7. A method according to any of the preceding claims, wherein the affinity specific molecule is selected from the group consisting of immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics, and lectins.
- 5 8. A method according to any of the preceding claims, wherein two or more different affinity specific molecules are present on particles within the adsorption medium.
9. A method according to claim 6 or 7, wherein the affinity specific molecules are selected from immunoglobulins.
- 10 10. A method according to any of the preceding claims, wherein the affinity specific molecule is Polymyxin B.
11. A method according any of the preceding claims, wherein the affinity specific molecule
15 is selected from the group consisting of a Toll-like receptor, most preferably TLR4 or binding fragments thereof or multimeric arrangements thereof, CD14, MD2, TLR2 and LBP, and any combination thereof.
12. A method according to any of the preceding claims, wherein the sedimented volume of
20 the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column.
13. A method for the treatment of sepsis caused by Gram-negative in a mammal by extracorporeal adsorption, said extracorporeal adsorption being effected by an adsorption
25 column assembly, said adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for the LPS portion of said Gram-negative bacteria, said method comprising the steps of:
- 30 d) obtaining blood from said mammal,
e) treating the obtained blood by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed,
and
35 f) reinfusing the treated blood into the same mammal.
14. A method for the treatment of sepsis caused by Gram-negative or Gram-positive bacteria in a mammal by extracorporeal adsorption, said extracorporeal adsorption being effected by an adsorption column assembly, said adsorption column assembly comprising a

WO 2004/008138

PCT/DK2003/000483

45

column and an adsorption medium in the form of particles, the sedimented volume of said particles being at the most 80% of the volume of the column, said particles being characterised by carrying an affinity specific molecule with a specific affinity for:

- 5 i) the LPS portion of said Gram-negative bacteria, and/or
- ii) Gram-positive bacteria or harmful substances derived from said Gram-positive bacteria, said method comprising the steps of:
 - 10 d) obtaining blood from said mammal,
 - e) treating the obtained blood by passing the blood through the adsorption column assembly at such a flow rate that a fluidised bed of the particles is formed, and
 - 15 f) reinfusing the treated blood into the same mammal.
15. The method according claim 13 or 14, wherein the flow rate of the blood through the column assembly is such that expansion ratio of the fluidised bed is at least 1.3, such as at least 1.5.
- 20 16. The method according to any of the claim 12-15, wherein the steps (a), (b) and (c) are preceded by a initial step by which a substance is first injected into the blood stream of the mammal.
- 25 17. The method according to any of the claims 13-16, wherein the mammal is a human being.
18. The method according to any of the claims 13-17, wherein the particles have a density of at least 1.3 g/ml and a mean diameter in the range of 5-1000 µm, such as a density of
- 30 at least 1.5 g/ml and a mean diameter in the range of 5-300 µm, preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 µm, and most preferred a density of more than 2.5 g/ml and a mean diameter in the range of 5-75 µm.
19. A method according to any of claims 11-18wherein the stabilised fluidised bed is
- 35 placed in line with a switch capable of being activated when a blood substance reaches a pre-set value, said blood substance is monitored by a device, said device is placed in line with the blood circulation, said device sending the activating signal to the switch when said value is reached.

WO 2004/008138

PCT/DK2003/000483

46

20. The method according to any of the claims 13-19, wherein the affinity specific molecule is selected from the group consisting of immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics, and lectins.
- 5 21. The method according to any of the claims 13-20, wherein two or more different affinity specific molecules are present on particles within the adsorption medium.
22. The method according to claim 20 or 21, wherein the affinity specific molecules are selected from immunoglobulins.
- 10 23. The method according to any of the claims 20 or 21, wherein the affinity specific molecule is Polymyxin B.
24. A method according to claims 13-23, wherein the affinity specific molecule is selected
15 from the group consisting of a Toll-like receptor, most preferably TLR4 or binding fragments thereof or multimeric arrangements thereof, CD14, MD2, TLR2 and LBP, and any combination thereof.
25. The method according to any of the claims 13-24, wherein the sedimented volume of
20 the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column.
26. The method according to any of the claims 13-25, wherein the flow rate is such that stabilised fluidised bed of the particles is formed.